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Biosynthesis of Silver Nanoparticles using *Carya Illinoinensis* Extract for Antibacterial Applications

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ABSTRACT

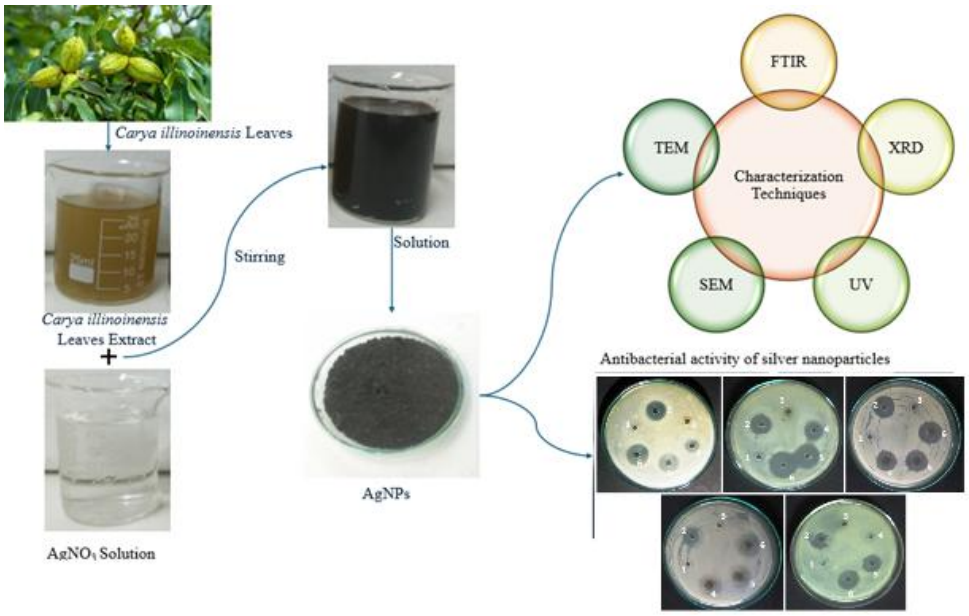
This study presents an eco-friendly approach for synthesizing silver nanoparticles (AgNPs) using *Carya illinoinensis* (pecan nut) leaves extract as a natural reducing and stabilizing agent. Extracts prepared at different reaction times were examined for protein, sugar content, and antioxidant activity. The 4-hour extract demonstrated the highest antioxidant capacity, indicating a greater concentration of reducing biomolecules suitable for nanoparticle formation. AgNPs synthesized using this

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extract and varying $AgNO_3$ concentrations produced a distinct color change, confirmed by UV–visible spectroscopy with a characteristic SPR peak at 398 nm. SEM and TEM analyses revealed predominantly spherical nanoparticles with sizes ranging from 2 to 60 nm, while EDX confirmed elemental silver as the primary constituent. FT-IR spectra indicated the presence of phenolic, polyphenolic, and protein-based functional groups on the nanoparticle surface, contributing to stabilization. Antibacterial assays showed strong inhibition zones against *Pseudomonas aeruginosa* (17 ± 0.50 mm), *Klebsiella pneumoniae* (14 ± 0.10 mm), *E. coli*, *Bacillus subtilis*, and *Staphylococcus aureus*, with activity increasing proportionally to nanoparticle concentration. The findings demonstrate that pecan leaves extract is a viable, low-cost, and sustainable source for producing bioactive AgNPs with significant antimicrobial potential.

Keywords: Green Synthesis, Silver Nanoparticles, *Carya Illinoinensis*, Antioxidant Capacity, Antimicrobial Activity.



Schematic Abstract

1. Introduction

Silver nanoparticles have emerged as one of the most extensively studied nanomaterials due to their distinct chemical, optical, electronic, and magnetic properties, which differ significantly from those of their bulk metallic forms[1]. Their unique physicochemical behaviour is strongly linked to nanoscale size effects and

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high surface-area-to-volume ratios, which enable a wide range of functional applications in medicine, environmental remediation, catalysis, electronics, and biosensing[2]. Among these applications, the antimicrobial activity of silver nanoparticles has attracted particular attention. Silver ions released from nanoparticles exhibit broad-spectrum antibacterial effects and remain effective against multidrug-resistant strains, which pose an increasingly serious threat to global public health[3-5]. Importantly, AgNPs have demonstrated strong microbicidal potential while generally maintaining low toxicity toward human cells, making them attractive candidates for the development of next-generation antimicrobial materials and coatings. Growing interest in nanomaterials has intensified the demand for sustainable, safe, and cost-effective synthesis routes. Conventional chemical and physical methods often involve high energy consumption and toxic reagents that generate hazardous byproducts, raising environmental and health concerns. In contrast, green synthesis offers an environmentally benign alternative by employing biological sources such as plants, microorganisms, and algae to reduce and stabilize metal ions[6-9]. Nanoparticles produced through biological systems are collectively described as biogenic nanoparticles. Green synthesis has attracted significant attention because biological extracts naturally contain molecules capable of reducing metal salts and stabilising the resulting nanoparticles without the need for harsh chemicals or elevated temperatures[10-12].

Plant-mediated synthesis has been particularly successful due to the abundance of secondary metabolites, including phenolic acids, flavonoids, alkaloids, terpenoids, proteins, and carbohydrates, which serve as reducing and capping agents. These compounds facilitate the conversion of ionic silver into elemental silver while stabilising the nanoparticles to prevent aggregation. The antioxidant capacity of plant extracts plays a crucial role in this process, and its evaluation provides valuable insight into the extract's ability to produce metallic nanoparticles. Extracts rich in phenolic compounds, for example, often demonstrate higher reducing power, leading to faster nucleation and smaller nanoparticle sizes. Among the various plant sources explored, *Carya Illinoinens* leaves have been previously used to synthesise silver nanoparticles due to their high phenolic content[12-15]. However, the *Carya Illinoinensis* Extract itself is an underexplored agro-industrial residue with considerable potential.

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The leaves contains substantial quantities of phenolic antioxidants and proanthocyanidins, including gallic, caffeic, and vanillic acids, as well as catechin and tannic acid. These compounds possess well-defined reducing properties and can play a crucial role in metal ion reduction and nanoparticle stabilisation. Valorising *Carya Illinoinensis* Extract waste for nanoparticle synthesis not only supports sustainable materials development but also contributes to the utilisation of agricultural byproducts that would otherwise be discarded[16-19].

Reaction conditions strongly influence the characteristics of biogenic nanoparticles. Factors such as precursor concentration, pH, temperature, and reaction time determine the size, morphology, and stability of the synthesized AgNPs. Higher concentrations of reducing agents have been associated with the formation of smaller nanoparticles due to enhanced nucleation. Reaction time affects growth kinetics, with prolonged incubation often yielding larger or more uniform particles depending on the extract composition. Solution pH alters the ionisation of functional groups in the extract and affects the reduction rate, leading to variations in size and shape. Similarly, higher temperatures accelerate nucleation and growth processes, often increasing the synthesis rate while influencing structural characteristics[20-22]. Understanding these relationships is critical for producing nanoparticles with controlled properties suitable for targeted applications. AgNPs produced through green synthesis have shown strong antimicrobial activity against both Gram-positive and Gram-negative bacteria as well as various fungal pathogens. Their ability to disrupt microbial membranes, interfere with respiration, and generate reactive oxygen species makes them valuable tools in the development of medical materials such as antimicrobial coatings, wound dressings, and drug delivery platforms[13-29]. Given the global rise of antibiotic resistance, the development of biogenic AgNPs with consistent antimicrobial performance holds significant promise for expanding eco-friendly strategies to combat infectious diseases[29-31].

The aim of this study was to obtain a plant extract rich in reducing biomolecules from *Carya Illinoinensis* leaves and to evaluate its potential for the green synthesis of AgNPs. To achieve this objective, the extract was characterised for its protein and carbohydrate content as well as its antioxidant capacity using DPPH,

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ABTS, and FRAP assays[34-36]. These analyses enabled a detailed understanding of the extract's reducing power and its suitability for nanoparticle synthesis. The influence of reaction parameters, including reaction time and the concentration of silver nitrate, was systematically investigated to determine their effects on nanoparticle size[37-38]. Furthermore, the antimicrobial activity of the synthesized AgNPs was evaluated against three pathogenic bacterial strains to confirm their applicability in medical and biological contexts. The findings of this work demonstrate that *Carya Illinoinensis* Extract is an effective reducing and stabilizing agent capable of producing AgNPs under mild, environmentally friendly conditions. The synthesis approach is cost-effective, straightforward, and rapid, offering advantages over conventional methods[39-40]. The resulting nanoparticles exhibit promising antimicrobial properties, highlighting their potential for applications in biomedical treatments, surface coatings, and other technological areas. This study contributes to ongoing efforts to advance sustainable nanotechnology by transforming agricultural waste into valuable functional materials[41-42].

2. Materials and Methods

2.1. Materials

Carya Illinoinensis leaves were collected from PMA Road, Abbottabad. Silver nitrate ($AgNO_3$, 99.5 percent purity) was obtained from Fermont. Bacterial cultures of *Salmonella typhi*, *Staphylococcus aureus*, and *Proteus mirabilis* were previously isolated from the Hospital General of Saltillo, Coahuila, and stored under laboratory conditions until use[43].

2.2. Preparation of *Carya Illinoinensis* Extract

The *Carya Illinoinensis* leaves were manually separated from the seeds, washed, and dried. The leaves were then ground using a ball mill (Fritsch Pulverisette 6, Idar-Oberstein, Germany) to obtain a fine powder, followed by sieving to 250 µm using Tyler mesh screens[24]. Aqueous dispersions were prepared by suspending one gram of leaves powder in 200 mL of distilled water with an initial pH of 6.5. Each suspension was heated at 80 °C under continuous magnetic stirring for 4, 8, and 12 hours, labelled as E1, E2, and E3. After heating, the mixtures were filtered through Whatman No. 4 paper to obtain the aqueous extract. Protein content was

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determined using the Kjeldahl method, while total sugars were quantified using the phenol–sulfuric acid method.

2.3. Antioxidant Capacity

The antioxidant capacity of each extract was evaluated using DPPH, ABTS, and FRAP assays following the procedures described by López-Contreras et al.[44] Results were reported as micromoles of Trolox equivalents per 100 g of sample, based on a standard calibration curve ranging from 0 to 500 µmol/L. All reagents used for these assays were procured from Sigma-Aldrich (St. Louis, MO, USA).

2.4. Green Synthesis of AgNPs

The extract exhibiting the highest content of reducing biomolecules was selected for nanoparticle synthesis. To examine the influence of precursor concentration and reaction time, $AgNO_3$ solutions at concentrations of 1 mM, 2 mM, and 4 mM were prepared. For each synthesis, 100 mL of $AgNO_3$ solution was placed in a round-bottom flask equipped with a condenser and heated to 80 °C with constant stirring. The selected plant extract was added, and reactions were maintained for 4, 8, or 12 hours. The formation of AgNPs was visually monitored through colour change and later confirmed using instrumental analyses.

2.5. Characterization of Nanoparticles

2.5.1. UV–Visible Spectroscopy

The optical properties of the synthesized AgNPs were examined using a UV–visible spectrophotometer (GBC Scientific, Cintra 2020, Hampshire, IL, USA) in absorbance mode. Spectra were recorded in the range of 200 to 600 nm to identify the characteristic surface plasmon resonance peak.

2.5.2. FT-IR Spectroscopy

Fourier transform infrared spectroscopy was used to identify the functional groups responsible for the reduction and stabilization of AgNPs. FT-IR spectra of the leaves extract powder and the synthesized nanoparticles were recorded between 4000 and 500 cm^{-1} using a Nicolet iS50 spectrometer (Waltham, MA, USA). Samples were prepared using the KBr pellet method.

2.5.3. Transmission Electron Microscopy (TEM)

Nanoparticle morphology and size distribution were analysed using transmission

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electron microscopy. A drop of the nanoparticle suspension was placed on a copper grid coated with a thin film and allowed to dry at room temperature. Imaging was performed on an FEI-Titan 80–300 kV TEM equipped with an energy-dispersive X-ray (EDX) analyser to confirm elemental composition.

2.5.4. Dynamic Light Scattering (DLS)

Particle size and hydrodynamic diameter were measured using a Zetasizer Nano Malvern S90 WR (Malvern, UK). Measurements were taken at 25 °C using distilled water at pH 6.5 as the dispersant. Each sample was analysed in triplicate to ensure reproducibility.

2.6. Antimicrobial Activity

The antimicrobial activity of the AgNPs was assessed using the disc diffusion method. Filter paper discs were impregnated with 100 µL of AgNP suspension and dried at room temperature. Bacterial inocula were prepared by adjusting cultures to 0.5 McFarland standard, corresponding to 1.5×10^8 CFU/mL. *S. aureus* was cultured on blood agar, while were cultured on Mueller–Hinton agar. Negative controls consisted of discs without nanoparticles. Discs were placed on inoculated agar plates with at least 3 cm spacing. Plates were incubated aerobically at 37 °C for 18–24 hours, after which inhibition zones were measured. All tests were performed in triplicate under sterile conditions.

2.7. Statistical Analysis

All experiments were conducted in triplicate. Data were analysed using analysis of variance (ANOVA), and significance was accepted at $p < 0.05$.

3. Results and Discussion

3.1. Protein Determination in *Carya Illinoinensis* leaves Extracts

The protein content of the *Carya Illinoinensis* Extracts remained below 1 percent across all reaction times, with measured values of 0.17 percent for the 4 h extract, 0.15 percent for the 8 h extract, and 0.17 percent for the 12 h extract (Table 1). Tukey's test showed no statistically significant differences among these means at $p < 0.05$, indicating that reaction time does not influence protein extraction under the conditions used. Previous reports indicate that *Carya Illinoinensis* leaves proteins typically contain essential amino acids such as arginine, lysine, and threonine, which

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may appear in low concentrations depending on the extraction conditions.

3.2. Sugar Determination in *Carya Illinoinensis* Leaves Extracts

Total carbohydrate content was quantified for each extract using the calibration curve associated with the phenol–sulfuric acid method. As shown in Table 1, increasing the reaction time resulted in a slight decrease in sugar concentration. This reduction may be attributed to thermal degradation of sugars when the extracts are exposed to 80 °C for extended periods, as well as the possible initiation of Maillard-type reactions. Despite this trend, ANOVA demonstrated that the differences were not statistically significant ($p < 0.05$), suggesting that prolonged extraction time does not markedly enhance or reduce sugar content.

3.3. Antioxidant Capacity of *Carya Illinoinensis* Leaves Extracts (ABTS, DPPH, and FRAP)

Antioxidant activity, expressed as micromoles of Trolox equivalents per 100 g of sample, is presented in Table 1 for the ABTS, DPPH, and FRAP assays. The extract obtained after 4 h exhibited the highest antioxidant capacity across all methods. These findings align with earlier work reporting high antioxidant potential in *Carya Illinoinensis* leaves, attributed to their abundance of phenolic compounds, flavonoids, and proanthocyanidins. In contrast, antioxidant activity declined as extraction time increased, likely due to the susceptibility of phenolic compounds to degradation under adverse conditions such as prolonged heat exposure, oxygen availability, and changes in pH or moisture. Statistical analysis revealed significant differences between ABTS and DPPH results, and between ABTS and FRAP ($p < 0.05$) across the extraction periods. Based on these outcomes, the 4 h extract was identified as containing the greatest concentration of reducing biomolecules and was therefore selected for AgNP synthesis. The results confirm that the aqueous extracts provide relevant biomolecules with notable antioxidant properties, and these structures collectively contribute to nanoparticle formation. Overall, while protein and sugar content did not vary significantly with extraction time, antioxidant capacity displayed a clear decline with longer processing. These results indicate that a reaction time of 4 h is optimal for obtaining extracts with the highest concentration of reducing compounds necessary for efficient AgNP biosynthesis.

Table 1: Protein, Carbohydrate Content And Antioxidant Capacity Of

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Carya Illinoinensis **Extract**

Sample	Proteins (%)	Carbohydrates (mg/g leaves)	ABTS (µmol TE/100 g)	DPPH (µmol TE/100 g)	FRAP (µmol TE/100 g)
E1 (4 h)	0.177 a	352.2 a	1262.6 b	154.9 b	322.4 b
E2 (8 h)	0.159 a	337.6 a	1095.6 a	99.7 a	107.4 a
E3 (12 h)	0.177 a	334.2 a	986.6 a	116.5 a	158.1 a

Note: *Means with a common letter are not significantly different ($p < 0.05$).*

3.4. Synthesis of AgNPs Using *Carya Illinoinensis* Leaves Extracts

The reaction between the Carya Illinoinensis's leaves aqueous extract, and the silver nitrate solutions produced a distinct brown–yellow coloration, as illustrated in Figure 1. The formation of AgNPs was visually evident through the noticeable change in solution colour before and after the reaction. Initially, the extract displayed a light-yellow tone, which gradually transformed into a dark brown colour following its interaction with $AgNO_3$. This colour transition served as a preliminary indicator of nanoparticle synthesis. Similar observations have been reported by Vijayakumar et al. (2013), who noted that exposure of plant extracts to silver ions leads to a progressive colour shift from pale yellow to brown. This visual change is attributed to the excitation of conduction-band electrons in the nanoparticles, a phenomenon known as localized surface plasmon resonance. When these electrons oscillate in response to incident light, specific wavelengths are absorbed while others are transmitted or scattered, producing the characteristic colour associated with AgNP formation.

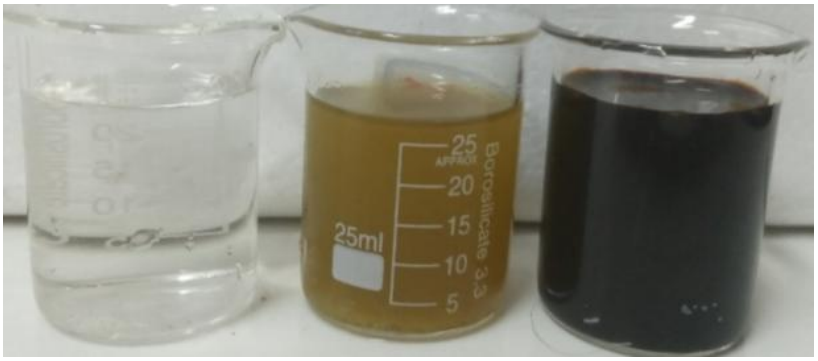


Figure 1. Appearance of Carya Illinoinensis leaves extract before and after

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reaction with $AgNO_3$

3.5. Characterization of AgNPs

3.5.1. UV-Visible Spectrophotometry

The formation of citrate-stabilized silver nanoparticles (AgNPs) was indicated by the distinct colour transition of the colloidal mixture to a yellow hue, as shown in Figure 1. This visual change reflects the initial reduction of silver ions and the subsequent emergence of nanoscale particles capable of interacting with incident light. To verify nanoparticle formation, UV–visible spectroscopy was performed. The resulting spectrum displayed a well-defined surface plasmon resonance (SPR) band at 398 nm, which is characteristic of AgNPs and confirms the presence of dispersed colloidal nanoparticles[35]. The appearance of this SPR peak is consistent with the collective oscillation of conduction electrons on the nanoparticle surface, demonstrating that the synthesis process successfully yielded citrate-stabilized silver nanoparticles.

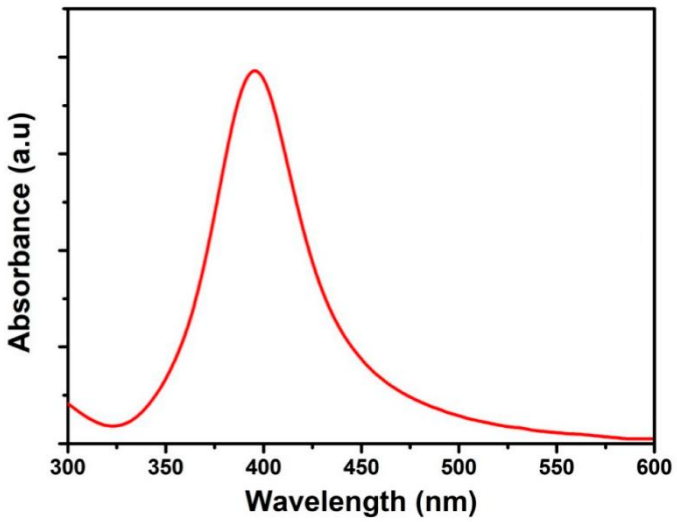


Figure 2: UV-VIS absorbance spectrum of the AgNPs synthetized by Carya Illinoinensis Extract

3.5.2. Scanning Electron Microscopy (TEM)

Scanning electron microscopy (SEM) was employed to examine the morphology and size distribution of the AgNPs synthesized using *Carya Illinoinensis* leaves extract. The micrographs (Figure 3) revealed that most of the nanoparticles were spherical, although a few particles exhibited irregular shapes. The bright spots visible in the

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images confirmed the successful formation of AgNPs. The nanoparticles showed a broad size distribution, ranging from 2 to 60 nm, which may be influenced by variations in particle shape[36]. The presence of larger particles could be attributed to the aggregation of smaller nanoparticles, likely promoted by residual moisture during sample preparation. These observations are consistent with previously reported findings in the literature.

3.5.3. Energy-dispersive X-ray (EDX)

Energy-dispersive X-ray (EDX) analysis further verified the elemental composition of the synthesized nanoparticles (Figure 4). A prominent peak near 3 keV, characteristic of silver due to its surface plasmon resonance, confirmed that Ag was the dominant element. Additional peaks at approximately 22 keV and 25 keV corresponded to AgL and Ag $K\alpha_1$/ $K\alpha_2$ lines. Signals detected at 8.0 and 9.01 keV were attributed to Cu Kα and Cu Kβ emissions from the copper grid used in TEM analysis. A minor peak around 0.7 keV indicated the presence of carbon, while C and Si signals may also reflect residual plant-derived compounds in the extract. Overall, these results confirm the successful reduction of silver ions to elemental silver, supporting the UV–Visible spectroscopy findings[37].

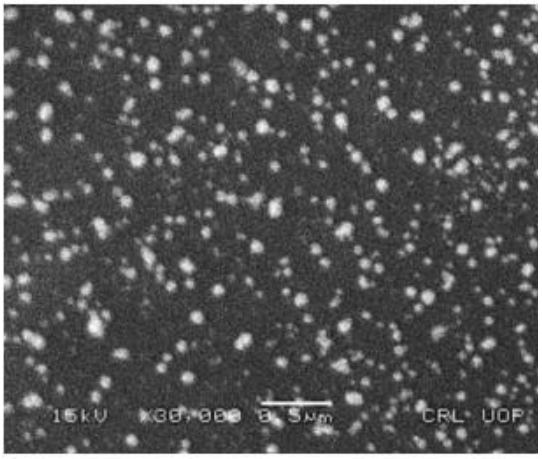


Figure 3: SEM photograph of AgNPs at 0.5 micron.

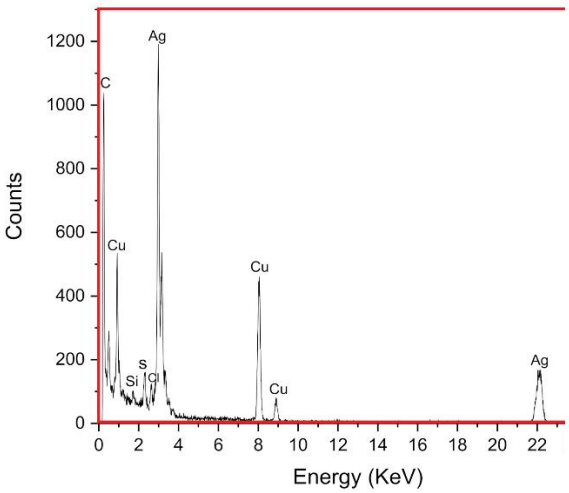


Figure 4. EDX spectrum of AgNPs synthetized by Carya Illinoinensis Extract.

3.5.4. Fourier Transform Infrared Spectroscopy (FT-IR)

Figure 5 presents the FT-IR spectra of the *Carya Illinoinensis* leaves aqueous extract

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(a) and the AgNPs synthesized from this extract (b). A broad absorption band at 3249–3268 cm^{-1} is associated with O–H stretching vibrations, which are characteristic of phenolic compounds present both in the extract and on the nanoparticle surface. In the AgNP spectrum, the band around 1709 cm^{-1} corresponds to carbonyl groups, indicating the presence of flavanones or terpenoids adsorbed onto the nanoparticles. These biomolecules likely interact with the metal surface through π-electrons of the carbonyl functional groups. Peaks observed at 1604–1612 cm^{-1} are attributed to N–H and C=O stretching of amines and amides, reflecting the protein content of the extract[38]. Absorption bands between 1007 and 1110 cm^{-1} correspond to phenolic and polyphenolic compounds originating from *Carya illinoinensis* components. Collectively, these features indicate that the AgNPs are stabilized by a coating of plant-derived biomolecules. A noticeable shift to 3412 cm^{-1} suggests alterations in hydrogen bonding, likely associated with the reduction of silver ions during nanoparticle formation. The overall similarity between the spectra of the extract and the AgNPs supports the conclusion that constituents of the extract form a protective leaves around the nanoparticles, contributing to their stability[39].

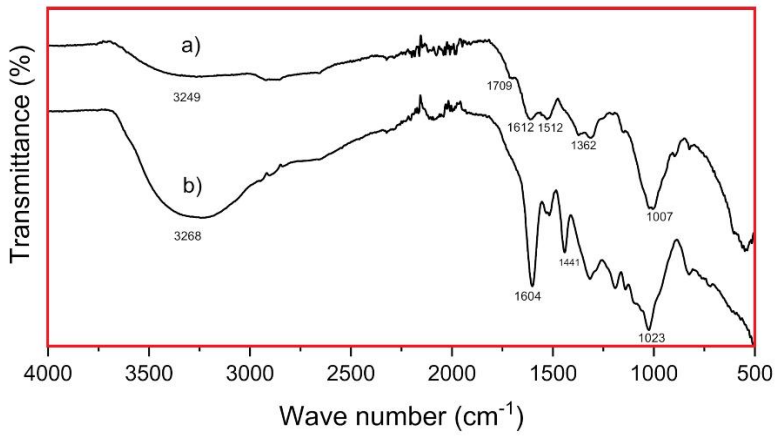


Figure 5. FT-IR of (a) silver nanoparticles and (b) Carya Illinoinensis Extract.

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3.6 Antibacterial Activity

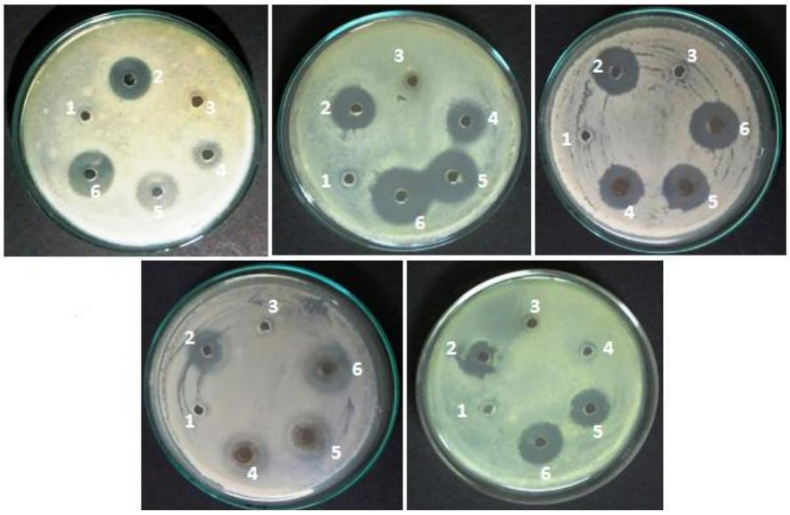


Fig. 6: Zone of inhibition produced by biosynthesized silver nanoparticles against various bacterial strains [D/W (Distilled water) (1), Silver nitrate (2), extract (3), AgNPs 10 µL (4), 30 µL (5), 50 µL (6)].

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Table 1: The results of antibacterial activity with zone of inhibition (mm) of *Carya Illinoinensis* synthesized silver nanoparticles against various microorganisms

Microorganisms	D/W	$AgNO_3$ (1 mM)	*Carya Illinoinensis* Extract	Synthesized Silver Nanoparticles (µL)		
				10 µL	30 µL	50 µL
Pseudomonas aeruginosa	--	7 ± 0.10	--	10 ± 0.33	12 ± 0.50	17 ± 0.50
E. coli	--	5 ± 0.30	--	5 ± 0.10	7 ± 0.10	11 ± 0.10
Bacillus subtilis	--	3 ± 0.10	--	5 ± 0.10	6 ± 0.30	8 ± 0.30
Staphylococcus aureus	--	5 ± 0.50	1 ± 0.10	5 ± 0.10	5 ± 0.50	7 ± 0.30
Klebsiella pneumoniae	--	6 ± 0.40	2 ± 0.10	7 ± 0.50	8 ± 0.30	14 ± 0.10

Note: "--" = No inhibition zone; SD = Standard deviation.

The antibacterial activity of *Carya illinoinensis*–mediated silver nanoparticles demonstrated a strong inhibitory effect against all tested bacterial strains, with inhibition zones increasing proportionally to the volume of AgNPs applied. As shown in Figure 6 and Table 1, distilled water and the plant extract alone did not exhibit antimicrobial activity, confirming that the observed inhibition is attributable to the biosynthesized nanoparticles. The $AgNO_3$ precursor solution displayed only mild activity, indicating that the reduction and stabilization of silver ions by phytochemicals in the extract plays a pivotal role in enhancing antimicrobial potency. Among the synthesized nanoparticles, the 50 µL concentration consistently produced the largest inhibition zones, particularly against *Pseudomonas aeruginosa* (17 ± 0.50 mm) and *Klebsiella pneumoniae* (14 ± 0.10 mm). These results highlight the strong bactericidal effect of AgNPs on Gram-negative species, which is notable given the structural complexity of their outer membranes. Similarly, moderate to strong inhibition was observed for *E. coli*, *Bacillus subtilis*, and *Staphylococcus aureus*, confirming the broad-spectrum potential of the synthesized nanoparticles. The superior activity of AgNPs compared to $AgNO_3$ may be attributed to the smaller size and larger surface area of the biosynthesized particles, which facilitate stronger interactions with bacterial cell membranes. The presence of phenolic and polyphenolic compounds from the extract likely contributes to nanoparticle stability

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and may synergistically enhance antimicrobial effects. The mechanism of action may involve disruption of cell walls, generation of reactive oxygen species, and interference with DNA and protein synthesis, as reported in previous studies. Overall, the results indicate that *Carya illinoinensis*–derived AgNPs possess significant antibacterial efficacy, demonstrating their potential for application in antimicrobial formulations, biomedical materials, and infection-control systems. Further studies on cytotoxicity and mechanistic pathways would strengthen their applicability for clinical and industrial use[44].

4. Conclusion

This study demonstrated that *Carya illinoinensis* extract is an effective and sustainable reducing agent for the green synthesis of silver nanoparticles. The extract obtained after 4 hours of processing showed the highest antioxidant capacity, indicating a richer concentration of reducing biomolecules essential for nanoparticle formation. FT-IR analysis confirmed the presence of phenolic, polyphenolic, and protein-based functional groups adsorbed onto the nanoparticle surface, contributing to stabilization and enhanced biological activity. SEM analyses revealed predominantly spherical nanoparticles with sizes ranging from 2 to 60 nm, while EDX confirmed silver as the primary elemental constituent. The synthesized AgNPs displayed strong antibacterial activity against all tested pathogenic bacteria, including *Pseudomonas aeruginosa*, *E. coli*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*. The inhibitory effect increased with nanoparticle concentration, with the 50 µL dose exhibiting the largest zones of inhibition. These results highlight the potential of *Carya illinoinensis*–derived AgNPs as broad-spectrum antimicrobial agents. The superior performance of biosynthesized nanoparticles compared to silver nitrate alone underscores the importance of plant-derived biomolecules in enhancing nanoparticle stability and biological effectiveness. This work confirms that *Carya illinoinensis*, an agro-industrial byproduct, can be successfully utilized to produce bioactive silver nanoparticles through an eco-friendly, low-cost, and efficient green synthesis approach. The findings support the applicability of these nanoparticles in biomedical, environmental, and antimicrobial technologies. Future investigations focused on cytotoxicity, mechanism of action,

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and formulation development will further advance the practical use of these green-synthesized AgNPs.

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